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Mutagensis of cloned DNA

Short Protocols Section 8

The classical approach to the study of the genetics of an organism or virus was to mutagenize the genome of the organism or virus using chemical or physical mutagens. Once the mutagenic events were completed and expressed, the changes in the genetic composition were studied by looking at changes in the phenotypic expression. These methods formed the basis for modern genetics. Unfortunately, the methods to mutagenize created random mutations rather than specific mutations in defined locations. In addition, more than one mutation per genome was common. Further, it was difficult to do fine structure analysis of the mutations due to the gross nature of the phenotype.

Recombinant DNA technology has enabled the development of a system to specifically define mutations and create whatever type of a mutation needed. The beauty of the system is that the complete genetic structure can be studied. For example, it is possible to determine if an entire sequence is necessary for a function, or if parts of the sequence are disposable, or if bases may be substituted. This carries fine structure mapping to a new level of definition.

Several approaches are described in section 8.

Oligonucleotide-directed mutagenesis without phenotypic selection. Kunkel procedure - Figure 8.1.1. In this procedure a defined mutation may be made at a defined site. The dut^-/ung^- strain used in the preparation of the template insures a high degree of success.

A random substitution may be made at a site by carefully programming the oligonucleotide synthesizer, Figure 8.2.1, 8.2.2. The purpose of this procedure is to create numerous mutations in a short DNA sequence. Once completed, then the sequence can be reinserted into the appropriate sites of the original DNA for in vitro and possibly in vivo testing.

Synthetic genes can be synthesized by annealing relatively long oligonucleotides by using overlapping ends. Then a complete gene can be synthesized. Figure 8.2.3. One advantage is that the sequence can be fitted to the purpose needed. For example, a gene normally expressed in *E. coli* could be placed behind the promoter sequences needed for expression in yeast or mammalian cells.

An alternative procedure to nucleotide specific mutagenesis is region specific mutagenesis Figure 8.3.1. In this procedure, the site to be mutated is subcloned into M13, or a ss plasmid, mutagenized, filled in and excised. Then the insert is cloned back into the original DNA and tested appropriately.

A restriction site can be introduced into a cloned fragment by PCR - Figure 8.5.1

A specific mutation can be introduced into a cloned fragment by PCR - Figure 8.5.2

With these types of experiments, it is essential to understand the sequence limitations on restriction enzyme digestion. NEB has done a careful study to determine the limits on a group of commonly used enzymes. -Table 8.5.1

Introduction of a point mutation by sequential PCR steps - Figure 8.5.3

These are powerful tools for studying the fine structure of genomes. The tools can also be used for the development of very powerful systems such as the modified M13 phages used for epitope mapping.